P-13 Interaction of B-cell Translocation Gene 2 with Adenine Nucleotide Translocator 2 in Granulosa Cells of Preovulatory Follicles of Rat Ovary

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Objectives: The present study was to examine the gonadotropin regulation and mechanism of action of BTG2 during the ovulation in immature rats.

Methods: Ovaries were also collected from immature (26-day-old) rats at various times after treatment with 10 IU PMSG for Northern blot, Western blot, and in situ hybridization analyses. Granulosa cells of preovulatory follicles were also collected by the method of follicular puncture using 23-gauge needles at different time intervals. Coimmunoprecipitation was utilized to confirm interaction of BTG2 with ANT2. To confirm colocalization of BTG2 and ANT2, subcellular fractionation was performed. GST pull down assay was performed to elucidate BTG2 domain interacting with ANT2 on modulating the function of ANT2 by generating BTG2 deletion mutants. ATP level in the preovulatory granulosa cells was determined by bioluminescence using a spectrofluorimeter with the ATP Bioluminescence HS II assay kit. Change of mitochondrial membrane potential difference was tested by FACS analysis with JC-1 stain of the living cells.

Results: Btg2 expression was transiently stimulated by LH/hCG in preovulatory granulosa cells. Interestingly, GST pull-down and coimmunoprec-ipitation assay demonstrated that Btg2 physically interacted with adenine nucleotide translocator (ANT) 2, a mitochondrial transmembrane protein. BTG2 and ANT2 proteins were colocalized in the mitochondrial fraction in transfected 293T cells. Deletion analysis of BTG2 demonstrated that the N-terminal end of Btg2 was responsible for interacting with ANT2 protein. Lastly, BTG2 modulated ANT2 functions on mitochondrial depolarization and ATP production.

Conclusion: In conclusion, Btg2 stimulated by LH/hCG interacts with ANT2, resulting in the modulation of ANT2 function and thus may exert its anti-proliferative role during the ovulatory process.

P-14 Egr1, a Critical Factor for Ovulation in the Mouse Ovary, is Transiently Induced by LH Signaling Pathway

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Objectives: The Egr family of zinc finger transcription factors consisting of 4 members regulates critical genetic programs involved in cellular growth, differentiation, and function. They are co-expressed in many different tissues, suggesting that they may have some redundant functions. Egr1(−/−) female mice showed infertility due to anovulation resulting from luteinizing hormone β subunit (LHβ) deficiency. While it is clear that Egr1 regulates transcription of LHβ in the pituitary gland, the roles of Egr1 in the ovary still remain unexplored. Thus, we have examined temporal expression profiles of Egr family and their cofactors (Nab1 and Nab2) in the ovary during ovulation process and whether Egr1 expressed in the ovary

is required for ovulation.

Methods: Immature Egr1(+/−) and Egr1(−/−) female mice (4 week old) were superovulated by intraperitoneal injection of PMSG followed by hCG 48 h later and paired with wildtype male mice. The numbers of 2-cell stage embryos and unfertilized oocytes collected from oviducts were counted at post-hCG 48 h, and viable embryos were cultured to blastocyst stage. Immature ovaries at various time points (post-hCG 0, 1, 3, 6, 12, and 24 h) during superovulation were collected for RNA extraction and/or histological analysis. Semi-quantitative RT-PCR with appropriate primers for Egr family and Nabs was performed.

Results: The number of oocytes superovulated from Egr1(−/−) mice was significantly lower than that of Egr1(+/−) mice. The rates of embryos developed to blastocyst stages were comparable between Egr1(+/−) and Egr1(−/−) mice. Egr1 mRNA was rapidly induced at post-hCG 1 h and its level reached peak at post-hCG 3 h with gradual decrease afterwards. While Egr1 was a predominantly expressed factor among Egr family in the ovary during ovulation, Egr2 and Egr3 had similar temporal expression patterns to that of Egr1, suggesting functional compensation for the loss of Egr1 in ovulation in the Egr1(−/−) ovary. Egr4 and Nabs were constantly expressed in the ovary throughout time points of superovulation.

Conclusion: Egr transcription factors are concomitantly and transiently induced by LH signaling pathways during ovulation process. Egr1 is an essential factor to fully respond to gonadotropins for ovulation in the ovary. The suboptimal response to exogenous gonadotropins for ovulation in Egr1(−/−) ovaries could be derived from functional compensation of other Egr family members in the ovary.

P-15 생쥐 Leydig Cell에서 Bisphenol A에 의한 성장조절인자의 변화

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Objectives: JAK (Janus family), ERK (MAP Kinase), STAT (Signal Transducers and Activators of Transcription) 등은 세포의 성장조절, 사멸, 줄기세포의 분화 그리고 병원성 물질에 대한 저항 기작과 관련한 cytokine 수용체계, 발암유전자의 발현 조절 등에서 중심적인 역할을 담당한다. 특히 JAK2와 ERK에서 STAT3 등으로 이어지는 신호전달은 GH등의 성장 인자에 의해 활성화되며 핵안의 여러 전사인자들을 조절하게 된다. 본 실험에서는 내분비계장애물질에 의한 생식기능에 있어 GH 및 IGF-1 신호전달의 변형을 이해하기 위해 GH 수용체를 경유한 주요 신호전달 구성요소인 JAK/.STAT 및 ERK 신호전달의 변형을 Leydig cell을 통해 확인하고자 한다. 또한 Leydig cell의 steroidogenesis에 있어서 내분비계장애물질의 처리에 따른 GH 작용의 교란도 확인하고자 한다.

Methods: 4주령의 생쥐 정소에서 Leydig cell을 원심분리법으로 분리, 수획한 후 10% FBS/DMEM media로 48시간 동안 배양하였다. 수세 후 GH, IGF-1, BPA를 각각의 농도 (10 ng/ml, 10 ng/ml, 0.1 nM)별로 처리하여 24시간 추가 배양하였다. 수획한 Leydig cell로부터 분리한 total RNA를 이용하여 최적화된 RT-PCR로 GHR mRNA와 steroidogenic enzyme 유전자의 발현량을 분석하였고, 단백질을 이용하여 Western blot을 수행하였다. Western blot은 SDS-PAGE 후 NC membrane에 전이시켜 ERK, JAK2, STAT3, 그리고 각각의 인산화 형태의 항체들을 사용하여 ECL kit로 신호를 검출하였다.

Results: GHR mRNA의 발현은 GH 처리시 증가하였고, GH와 BPA를 동시에 처리하였을 때는 감소하였다. steroidogenic enzyme 유전자의 경우 GH 처리시 3β-HSD와 StAR mRNA 발현이 증가하였고, GH와 BPA 동시 처